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Emergence and dispersal of snakes after syntopic hibernation

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Abstract

A communal hibernaculum situated in Southwestern Bulgaria and its adjacent area (4 km in diameter) was studied. Seven snake species used this hibernating den, *Malpolon insignitus*, *Dolichophis caspius*, *Platyceps najadum*, *Elaphe quatuorlineata*, *Telescopus fallax*, *Vipera ammodytes* and *Xerotyphlops vermicularis*. The emergence of the snakes was related to the rise of daily temperatures. *Malpolon insignitus* emerged from hibernation earlier in the spring than the other species, and most individuals were the first to leave the hibernaculum. There was a temporal segregation among the basking area in early spring, between the latter species and *D. caspius*, which were the two most abundant species. These two species also used the same area of the hibernaculum, in contrast to *P. najadum* and *E. quatuorlineata*, which used a different area. After spring dispersal, *M. insignitus* and *D. caspius* used the same microhabitats during the rest of the active period. The earlier emergence of *M. insignitus* (and earlier departure from the site) probably reduces the competition for basking sites. The early departure of the species may give advantage in the competition for optimal microhabitats with *D. caspius*.

Key Words

microhabitat, hibernaculum, competition, segregation, Colubridae

Introduction

Snakes living in high latitude areas spend part of the year in hibernation, avoiding low winter temperatures. In these circumstances, mass congregations of snakes in mono- or heterospecific groups around hibernacula are common (Parker and Brown 1973; Gregory 1977, 1984). The selection of the appropriate hibernaculum is crucial for the winter survival of the snakes (Viitanen 1967; Prestt 1971; Gregory 1977; Altwegg et al. 2005). Thus, communally hibernating snakes tend to exhibit a high degree of fidelity towards the overwintering den (Hirth 1966; Brown and Parker 1976; Gregory 1977; Brown 1992), with individuals sometimes migrating several kilometers from summer habitats to the dens (Viitanen 1967; Gregory 1984; Gannon and Secoy 1985; Graves and Duvall 1993). Most studies on hibernation focus on the physiological adaptations associated with it (Aleksiuk 1970, 1976; Congdon et al. 1979; Crews et al. 1987; Costanzo 1989; Ultsch 1989; Graham and Graham 1992; Gregory 2011), thermal regimes of the hibernacula and temperatures of snakes during hibernation (Brown et al. 1974; Jacob and Painter 1980; Taylor and Nol 1989; Weatherhead 1989; Harvey and Weatherhead 2006) or the links between the duration of hibernation and environmental factors (Sexton and Hunt 1980; Sexton and Marion 1981; Brent Charland 1989; Macartney et al. 1989; Grobman 1990; Crawford 1991; Blouin-Demers et al. 2000). Fewer studies focus on the activity and spatial ecology of snakes in proximity to the dens following spring emergence (Viitanen 1967; Prestt 1971; Graves and Duvall 1990; Meek 2014; Rugiero et al. 2018). After emergence, snakes often

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use the vicinity of the hibernacula for thermoregulation and reproductive activity (Viitanen 1967; Prestt 1971; Gregory 1984; Phelps 2004; Meek 2014).

In Bulgaria, snakes' hibernation usually lasts from late October to late March or early April (Stojanov et al. 2011). The southwestern part of the country, along the Struma River, is species-rich in reptiles with a total of 12 snake species found in partial or full sympatry (Petrov and Beshkov 2001; Stojanov et al. 2011). In the present study we describe activity patterns and the spatial dynamics of several snake species using the same hibernating den.

Material and methods

The study area is located in Southwestern Bulgaria, in the vicinity of the town of Kresna (41°43'N, 23°10'E (DDM); 180 m a.s.l.). The hibernaculum is an abandoned old stone wall building, partially dug into the foot of a small hill. It is surrounded by an area of about 1300 m^2, which the snakes use for basking after the spring emergence (Fig. 1). The area is comprised of stone piles and stone walls, surrounded by grass vegetation, sporadic shrubs and single trees. The area adjacent to the hibernaculum (within a 2 km radius) is dominated by agriculture (vegetable plantations and vineyards), surrounded by irrigation canals and hills, overgrown with grass and shrub vegetation.

Sporadic visits to the hibernaculum and adjacent area were made during spring and autumn from 2012 to 2017. The visits were made once or twice per month, in suitable weather conditions, and lasted one day, from morning to late afternoon (see Table 1). In the spring of 2018 the hibernaculum was visited almost daily from 1 March to the beginning of May, with the duration of each visit lasting from morning to late afternoon. No visits were made on very rainy days, or on dry but cold days, with temperatures below 10 °C. From the beginning of March to the first half of April, the adjacent area of the hibernaculum (radius of 250–300 m) was also surveyed. After the first half of April, when all snakes left the hibernaculum, the surveys covered a larger area extending the search radius to 2 km from the hibernaculum. The difference in the search perimeter between early spring and the second half of April was due to the seasonal differences in movement rates in snakes. Following spring's emergence, snakes exhibit a tendency to remain in close proximity to the hibernating area, lasting until the start or the end of the mating period, when they can disperse, sometimes several kilometers away from it (Viitanen

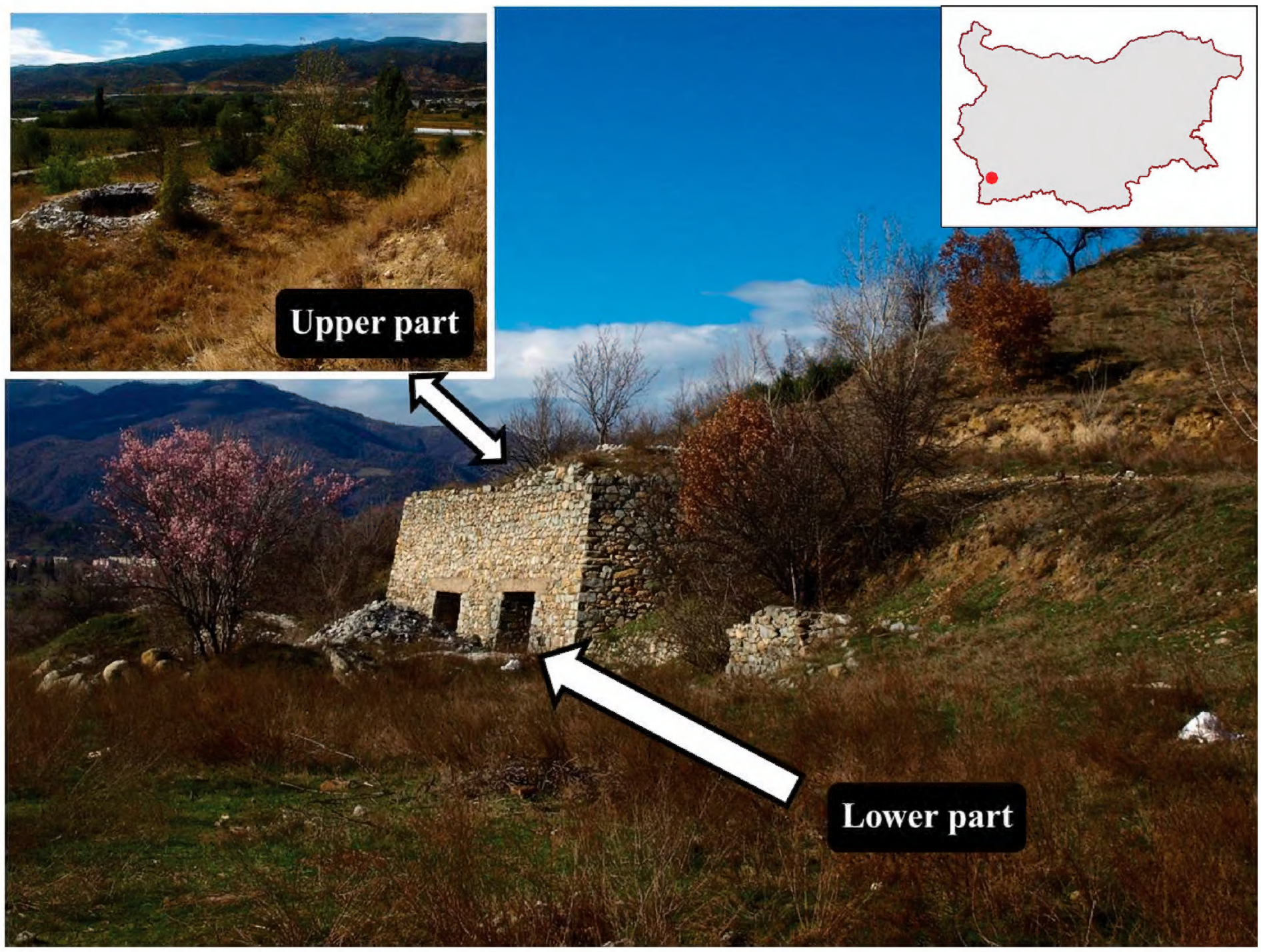

Figure 1. View of the studied hibernaculum with the upper (small left picture) and lower basking area. In the right small picture – map of Bulgaria with the location of the study site (filled dot).

1967; Prestt 1971; Ciofi and Chelazzi 1994; Phelps 2004; Meek 2014). Several night visits to the hibernaculum and adjacent area were also made in spring, but they usually lasted no more than 1.30 hours. In June, July and August 2018, the hibernaculum and the adjacent area were visited once a month for two days. From 25 September to 11 October 2018 and from 26 to 30 October 2018, the hibernaculum and the area around it were again visited daily.

Commencing from the sighting of the first snake outside the hibernaculum in 2018, air temperatures 15 cm above the ground, as well as the temperatures of the grass and the rocks was measured, using a digital thermometer (Allosun ETP109, with accuracy to ± 0.3 °C). These measurements were carried out three times a day (between 12:30–13:00, 14:00–14:30 and 15:30–16:00 hours) during all visits until all the snakes left the hibernaculum, and were always measured in the same spot. The average of the air, grass and rock temperature was used to define microhabitat temperature (Tmh); and Tmh were averaged daily. Snakes were located by visual observation during active searches along with inspections of potential hideouts e.g., holes, burrows and rock crevices as well as flipping stones and logs. For every observed snake, the date, species, time, position around the hibernaculum and the activity were recorded. The microhabitat of the location was classified according to the percentage of trees, shrubs, grasses, stones and rocks, water surfaces and roads (with total of 100 %) within a radius of 2.5 m from the snake location. If possible, snakes were captured, measured, weighed and photographed. In 2018, all captured snakes were permanently marked, using PIT-tags (ISO11784 FDX-B). The individuals, captured at the hibernaculum in 2018, were also marked with a non-toxic, alcohol-free color pen (Faber-Castell Multimark 1525 permanent) to avoid capture and disturbance of already marked individuals. If possible, the sex of the snakes was determined by inspection of the tail morphology, as well as by eversion of the hemipenis where possible. After all procedures were carried out, the snakes were released at the place of capture. For snakes found in the adjacent area, coordinates were taken with a hand-held GPS device (Garmin eTrex 20, with accuracy to ± 10 m) and the date, species, time, activity and the microhabitat were described. Captured snakes were measured and marked with PIT-tags. The air temperature 15 cm above ground and the temperature of the substrate on which the animal was found were measured.

The period of emergence, observed around the hibernaculum in the spring of 2018, was calculated as the number of days between our initial visit to the hibernaculum (1 March) and the observation date of each individual in the locality. Observations of the same individual but on different days were treated as different observations, in order to evaluate not only the dates of emergence, but the time spent around the hibernaculum. For analysis of the emergence periods and the relations between the temperatures and the activity of the snakes, only the data from spring 2018 were used, while data across all years were used for the analysis of the utilization of the different sections of the hibernaculum by different species and for microhabitat analysis both near and away from the hibernaculum. The abundance (*Ab*) of *M. insignitus* and *D. caspius* in the adjacent area was calculated by the formula: $Ab = N/H$, where N is the number of observations for this species, and H is the search time in hours. For this calculation the data from all years for snakes, observed only outside of the basking area of the hibernaculum, were used. Since only three specimens of *V. ammodytes* and no specimens of *P. najadum* and *E. quatuorlineata* were found outside the basking area throughout the active season, the abundance of the latter three species was not calculated.

Most of the data were not normally distributed (Shapiro-Wilk W test), therefore non-parametric tests were used. For analyses of the Tmh during days of emergence and ingress, a Mann-Whitney U test was used. Spearman's correlation was used to evaluate the connection between Tmh and number of emerged individuals and species. A χ^2 test was used for comparing the presence in March and April 2018 of the different species on the basking area. A correspondence analysis, based on a frequency matrix, i.e., the number of observations for each species on the upper and lower parts of this area in March and April, was also used. Kruskal–Wallis ANOVA was used for the analyses of the periods of emergence of the species, as well as for the microhabitat characteristics. The percentage covarage of trees, water bodies and roads were very low, and not included in the analysis. All statistics were performed using Statistica 10.0 (StatSoft, Inc. 2011).

Results

A total of seven species used the hibernaculum: *Xerotyphlops vermicularis* (Merrem, 1820), *Platyceps najadum* (Eichwald, 1831), *Dolichophis caspius* (Gmelin, 1789), *Elaphe quatuorlineata* Bonnaterre, 1790, *Malpolon insignitus* (Geoffroy de St-Hilaire, 1827), *Telescopus fallax* Fleischmann, 1831 and *Vipera ammodytes montandoni* Boulenger, 1904. Due to the specific biology of *X. vermicularis*, it was not included in the analyses. Only one juvenile of *T. fallax* was found in October 2018, so this species was also not included. *Natrix natrix* (Linnaeus, 1758), *Natrix tessellata* (Laurenti, 1768) and *Eryx jaculus* (Linnaeus, 1758) were found in the adjacent area close to the hibernaculum (within a radius from 100 to 600 meters), but were not observed in the immediate vicinity. The preliminary observations on the hibernaculum (18 visits before 2018) are summarized in Table 1.

In 2018, the first snakes emerged from hibernation in the middle of March (12, 13 and 15). Following initial emergence, an ingress back to the hibernaculum was observed, lasting from 16 to 27 March. During this period no snakes were observed. A second period of emergence started on 28 March and lasted until 13 April. After 13 April, no snakes were observed around the hibernaculum (Fig. 2). The microhabitat temperatures (Tmh) during the two periods of emergence were higher than those

Table 1. Number of individuals per species for each visitation before 2018. Asterisk denotes one and the same individual. *X. v.* is *Xerotyphlops vermicularis*; *D. c.* is *Dolihophis caspius*; *P. n.* is *Platyceps najadum*; *E. q.* is *Elaphe quatuorlineata*; *V. a.* is *Vipera ammodytes*.

Date	*X. v.*	*D. c.*	*P. n.*	*E. q.*	*V. a.*
2012 May 10	–	–	–	–	–
2013 April 6	–	4 ad.	2 ad.	–	1 ad male*
2013 May 27	–	–	–	–	–
2013 May 31	–	–	–	–	–
2013 Sept 18	–	–	–	–	–
2013 Sept 19	–	–	–	–	–
2013 Oct 22	–	–	–	–	–
2014 March 18	–	2 ad.	2 ad.	1 juv.	1 ad male*
2014 May 5	–	–	–	–	–
2014 Oct 22	–	–	–	–	–
2016 March 19	–	–	1 subad.	–	–
2016 April 23	1 ad.	–	1 ad.	–	1 ad male*
2016 April 25	–	–	1 ad.	–	1 ad male*
2017 March 21	–	2 ad.	1 ad.	–	1 ad female
2017 April 16	–	–	–	1 ad.	–
2017 April 29	–	–	–	–	–
2017 Sept 25	–	–	–	–	–
2017 Oct 22	–	–	–	–	–

during the ingress period, with a statistically significant difference (period of emergence: means ± SD: 21.95 ± 2.86 °C, range: 16.02–27.29 °C, $N = 16$; period of ingess: means ± SD: 16.27 ± 3.2 °C, range: 11.9–21.37 °C, $N = 8$; Mann-Whitney U test: $U = 13.00$, $p = .002$) (see Fig. 2). Also, positive correlations were found between the temperature of the day and both the number of snakes found around the hibernaculum (Spearman's correlation: $r = .61$, $p < .01$, $N = 24$) and the number of species found (Spearman's correlation: $r = .69$, $p < .001$, $N = 24$).

Malpolon insignitus was the first species to emerge from hibernation, with two individuals, an adult and a subadult, observed on 12 and 13 March (Fig. 2). On 28 and 29 March two different specimens were observed: a subadult specimen, observed on 28, and an adult, observed on 28 and 29. A different adult specimen was observed on 8 and 11 April. The first emergence of *P. najadum* was recorded on 15 March, an adult male. The same specimen was observed again on 30 March and 1 April, and on 3 and 5 April. On 4 and 5 April, a different specimen of *P. najadum* was observed. A final sighting of *P. najadum* was made on 8 and 12 April, but capture was not possible so its identity could not be determined. *Dolichophis caspius* was observed first on 28 March, an adult specimen. From 1 to 8 April numerous specimens were observed, with a peak on 5 April, five different individuals. Only a few of the specimens were captured, so individual identification is uncertain. *Elaphe quatuorlineata* was initially found on 30 March- an adult male. The same specimen was observed again on 4 April. An adult specimen *E. quatuorlineata* was observed again on 5 April, but was not captured (its identity not determined). *Vipera ammodytes* was the final species to emerge in 2018, with a single adult female specimen, observed on 11, 12 and 13 April. This was the same individual caught on 21 March 2017 (see above).

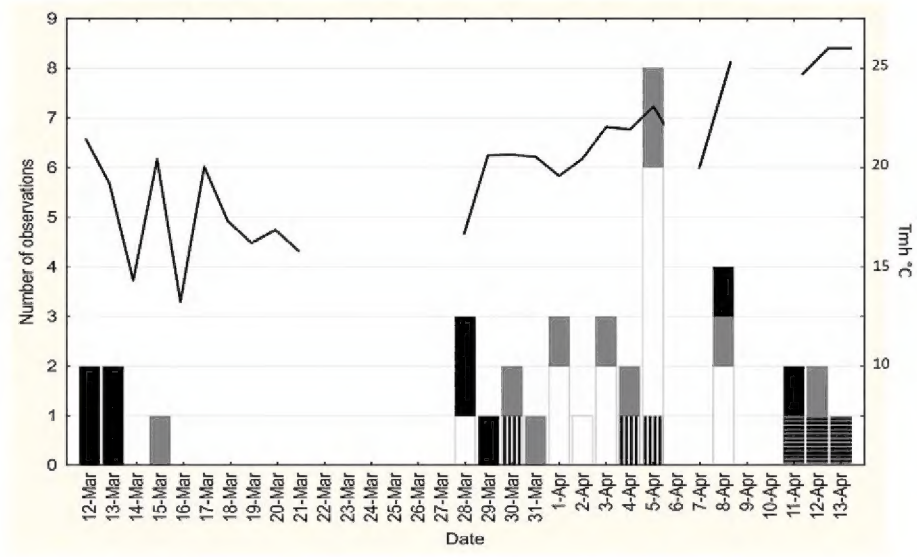

Figure 2. Dates of observations on the different species at the basking area of the hibernaculum during spring 2018. The figure represent the period between the first and the last date on which snakes were found there. Black bars represent *M. insignitus*, grey, *P. najadum*, white, *D. caspius*, vertically striped, *E. quatuorlineata*, horizontally striped, *V. ammodytes*. The black line presents the mean daily temperature of the microhabitat (Tmh) in days of visitation.

The descriptive statistics of the emergence periods for the species are given in Table 2. The period of emergence differed significantly between species (Kruskal-Wallis ANOVA; $H = 12.69$, $N = 38$, $p = .01$). However, post-hoc analysis showed that only the difference between *M. insignitus* and *V. ammodytes* was significant (Multiple comparisons post hoc test; $p = .006$). A statistically significant difference was found between the presence of *M. insignitus* and *D. caspius* in the basking area of the hibernaculum in spring, with most of the individuals of *M. insignitus*, being observed in March, and those of *D. caspius*, in April (χ^2 with Yates' correction = 8.46, DF = 1, $p < .01$). No statistically significant difference was found between the presence of *M. insignitus* and *P. najadum* ($\chi^2 = 2.63$, DF = 1, $p = .1$) or between *P. najadum* and *D. caspius* ($\chi^2 = .71$, DF = 1, $p = .39$); due to the low number of observations, *E. quatuorlineata* and *V. ammodytes* were not included in this analysis.

A spatial segregation among the species was established with regard to utilization of the basking area of the hibernaculum (lower/upper part; see Fig. 1). *Malpolon insignitus* and *D. caspius* basked mainly at the lower part of the hibernaculum: respectively 78% of the observed specimens ($N = 9$) and 100% ($N = 12$). *Platyceps najadum* and *E. quatuorlineata* basked mainly in the upper part: 89% ($N = 18$) and 80% ($N = 5$) respectively. In the case of *V. ammodytes* some variability in basking position was observed (lower part 63%, upper part 37%, $N = 8$), due to the changing of the basking site of the female viper in 2018. In 2017 this viper basked on the lower part of the hibernaculum and in 2018, in the upper part. In contrast, the male specimen, observed from 2013 to 2016, was always observed basking in the lower part. Correspondence analysis illustrates spatial-temporal distribution of the collected data relatively well (first two axes explain more than 95% of the variance). Site location of the species and the four combinations for using the hibernaculum (lower and upper parts in March and April) in the space of the

Table 2. Periods of emergence and values of Tmh for the species on the basking area of the hibernaculum. The period of emergence is calculated as the number of days between the initial visitation of the hibernaculum (1 March) and the observation date of every specimen from that species on its vicinity. *N* represents the number of observations of different individuals from the species. Observations of the same individual but on different days are treated as different observations.

Species (*N*)	Period of emergence [days]		Temperature of microhabitats [°C]	
	Mean ± SD	Min–Max (range as dates)	Mean ± SD	Min–Max
M. insignitus (9)	24.00 ± 11.92	12–42 (12 March – 11 April)	20.39 ± 3.16	16.02–25.33
P. najadum (10)	33.10 ± 7.43	15–43 (15 March – 12 April)	22.55 ± 2.23	19.55–26.43
D. caspius (13)	34.69 ± 3.01	28–39 (28 March – 08 April)	22.29 ± 2.68	16.02–25.33
E. quatuorlineata (3)	33.67 ± 3.21	30–36 (30 March – 05 April)	22.32 ± 1.46	20.82–23.75
V. ammodytes (3)	43.00 ± 1.00	42–44 (11 April – 13 April)	25.75 ± 1.96	23.54–27.29

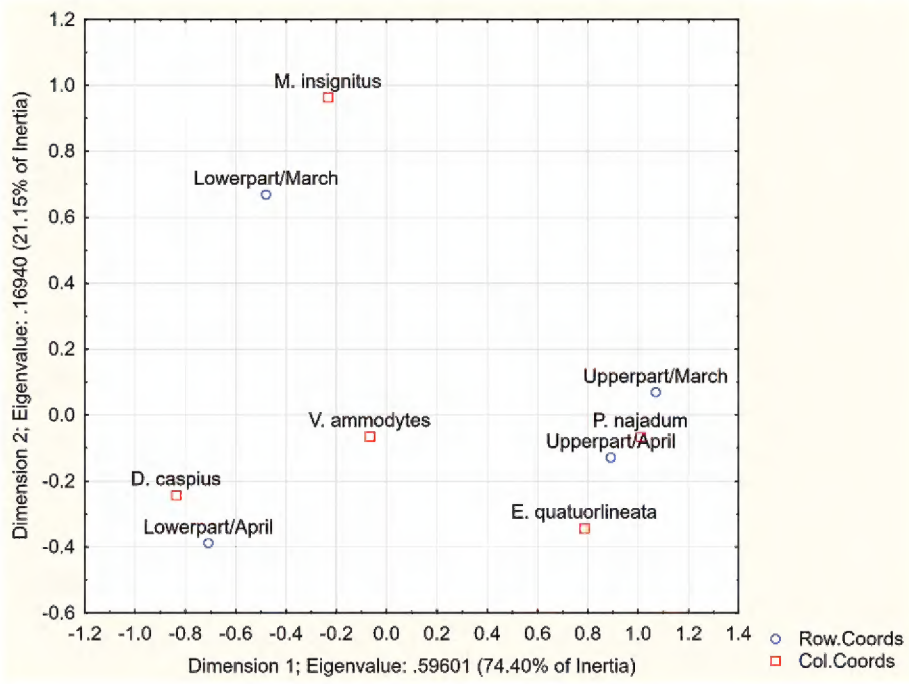

Figure 3. Distribution of the species and the basking area/month described by the correspondence analysis.

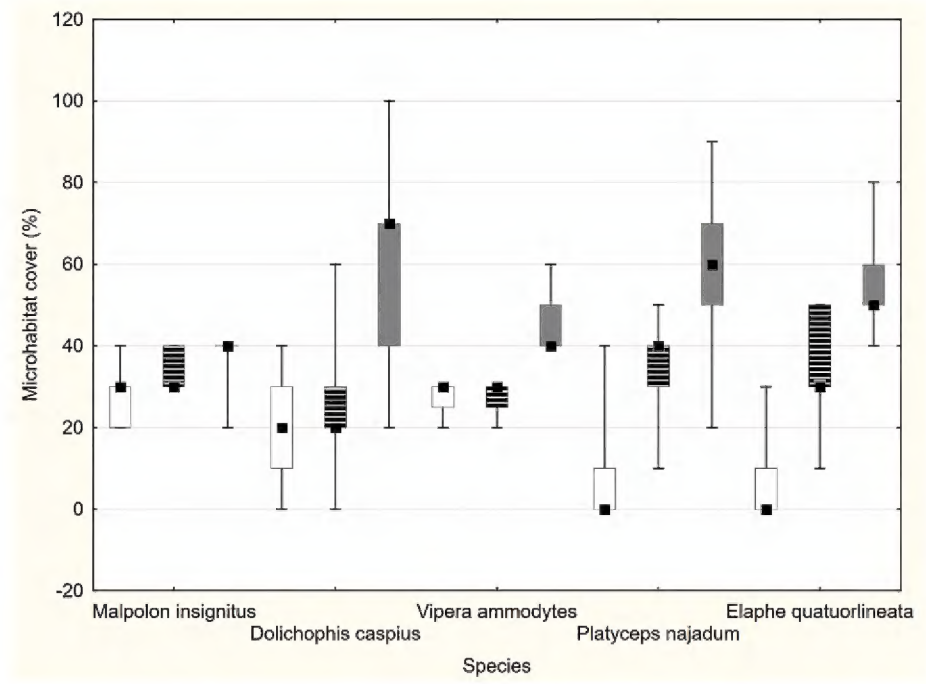

Figure 4. Microhabitat cover of the basking areas around the hibernaculum. The microhabitat is represented by the percentage of shrubs (white box), grass (horizontal striped box) and stones+rocks (dark grey box) with a total sum of 100. The graph is presented by median (black squares), 25–75 percentiles (boxes) and range (whiskers).

first two axes show (Fig. 3) that *M. insignitus* was associated with the lower part in March and *D. caspius* with the lower part in April; *P. najadum* and *E. quatuorlineata* were associated with the upper part of the hibernaculum, but not with a specific month; *V. ammodytes* (very low values on both axes) could not be related to either of the spatial-temporal combinations of hibernaculum use.

With regard to the temperature of the microhabitat (see Table 2), it appears that *M. insignitus* was found active at lower Tmh than *P. najadum*, *D. caspius* and *E. quatuorlineata*, and, on the other hand, that *V. ammodytes* was found at higher Tmh, but the differences were not statistically significant (Kruskal-Wallis ANOVA: $H = 7.83$, $p = .09$), however the p value is close to the 95% interval required for significance.

Some differences between species were found also in regard to the type of basking microhabitat (see Fig. 4). *Platyceps najadum* and *E. quatuorlineata* basked in predominantly stony microhabitats with grass vegetation, and very low presence of shrubs. *Dolichophis caspius* and *V. ammodytes* also used predominantly stony microhabitats, but the presence of shrubs was higher and equal to that of the grasses. *Malpolon insignitus* was seen basking mainly on microhabitats with equal cover of stones, grass and shrubs. Statistically significant differences between the species with regard to microhabitat land cover (see Table 3) were established as follows: for the shrubs occu-

Table 3. Post-hoc results, showing the p values from the Kruskal-Wallis test for the microhabitat cover of the species in the basking area of the hibernaculum. *M. i.* is *Malpolon insignitus*; *P. n.* is *Platyceps najadum*; *D. c.* is *Dolihophis caspius*; *E. q.* is *Elaphe quatuorlineata*; *V. a.* is *Vipera ammodytes*.

Shrubs cover (Kruskal-Wallis ANOVA; $H = 23.64$, $p = 0.0001$)				
	M.i.	***P.n.***	***D.c.***	***E.q.***
P.n.	0.003			
D.c.	1	0.1		
E.q.	0.07	1	0.87	
V.a.	1	0.003	1	0.08
Grass cover (Kruskal-Wallis ANOVA; $H = 17.20$; $p = 0.002$)				
	M.i.	***P.n.***	***D.c.***	***E.q.***
P.n.	1			
D.c.	0.1	0.005		
E.q.	1	1	0.76	
V.a.	0.75	0.19	1	1
Rock and stone cover (Kruskal-Wallis ANOVA; $H = 12.99$; $p = 0.01$)				
	M.i.	***P.n.***	***D.c.***	***E.q.***
P.n.	0.046			
D.c.	0.03	1		
E.q.	0.31	1	1	
V.a.	1	1	0.75	1

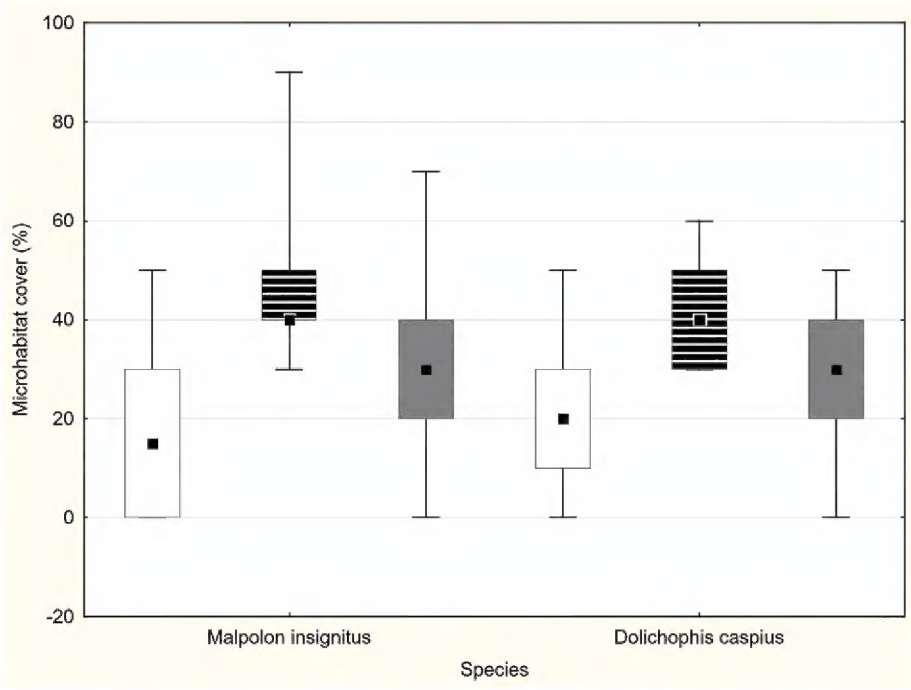

Figure 5. Microhabitat cover of the places of observation of *M. insignitus* and *D. caspius* following dispersal from the hibernaculum. The microhabitat is represented by the percentage of shrubs (white box), grass (horizontal striped box) and stones+rocks (dark grey box) with a total sum of 100. The graph is presented by median (black squares), 25–75 percentiles (boxes) and range (whiskers).

pancy between *P. najadum* and *M. insignitus* and between *P. najadum* and *V. ammodytes*; for the grass occupancy only between *P. najadum* and *D. caspius*; for the rock and stone occupancy between *M. insignitus* and *P. najadum* and between *M. insignitus* and *D.caspius*. The sample size for *E. quatuorlineata* was too low for analysis.

After 13 April no snakes were observed on the basking area of the hibernaculum until the beginning of October. However, we found moult from *D. caspius* and *P. najadum* around the hibernaculum between the end of June and the first half of July, at the end of August and at the end of September. Throughout the years only three moults of *M. insignitus* were found around the hibernaculum, all of them at the end of September. Outside the basking area of the hibernaculum *D. caspius* and *M. insignitus* were equally abundant during the active season ($Ab = .77$ and $Ab = .72$ respectively, total search hours $N = 195.35$). Throughout the active season these species used the same microhabitats (Fig. 5): both were found in predominantly grassy microhabitats with stones and scattered shrubs. Kruskal-Wallis ANOVA showed no statistically significant difference between the two species (shrubs: $H = .98$, $p = .32$; grass: $H = .1$, $p = .75$; stones and rocks: $H = .01$, $p = .91$). We were not able to observe *P. najadum* and *E. quatuorlineata* after the dispersal from the hibernaculum. Three specimens of *V. ammodytes* were found throughout the active season in the adjacent area, none of which had been previously observed in the hibernaculum.

In autumn, only two snakes were found around the hibernaculum, an adult female *E. quatuorlineata* and a juvenile *T. fallax* (1 and 11 October respectively).

Discussion

Although the hibernaculum was not visited regularly, there appears to be a variation in the timing of spring emergence. In 2014, we observed six specimens on the basking area in the middle of March, belonging to four species. The variation of the timing of emergence was even more obvious in 2017, when the female *V. ammodytes* was observed in the second part of March, 20 days earlier than in 2018 as well as two *D. caspius* and a *P. najadum*. It should be noted that the winter of 2016/2017 was warmer than usual, and in other, more northern parts of Bulgaria we observed the first emerged snakes at the end of February (A. Dyugmedzhiev, unpublished data). Spring emergence in snakes usually starts after a few days of warm and mild weather, with temperatures above 15–16 °C (Macartney et al. 1989; Capula et al. 1997), which is consistent with our observation at our study site where the first sightings in 2018 were on days with temperatures above 16 °C. Most of the days from 3 March to the day the first snake emerged, were fairly warm, with temperatures around 12–15 °C. Spring emergence is believed to be triggered by the increase in outside temperatures, reversing the thermal gradient in the hibernaculum, with temperatures higher around the surface levels (Viitanen 1967; Jacob and Painter 1980; Sexton and Hunt 1980; Sexton and Marion 1981; Costanzo 1986; Blouin-Demers et al. 2000). Our data suggests emergence from the den was due to increases in daily temperatures.

Based on identification of individual snakes site fidelity for use of the hibernaculum is indicated. For example, the male viper was observed on the same spot from 2013 to 2016. The female viper was also observed in two consecutive years on the hibernaculum, although in different basking spots. Frequent use of the same basking spots of the specimens per species, throughout the years, suggests den fidelity for hibernation as seen in other vipers (Luiselli et al. 2018). Selecting the hibernation den is crucial for the survival of the snakes during winter and communally hibernating snakes tend to use the same hibernating sites every year (Hirth 1966; Brown and Parker 1976; Gregory 1977; Brown 1992).

The spatial segregation among the species, observed in the basking area, indicates that some, if not all, of the different species, and probably even some individuals, hibernate separately. The underlying causes for selection of different parts of the hibernaculum used by different species are unclear. One plausible explanation is that this separation prevents the overcrowding both in the den during winter and in the basking area during spring, thus lowering the competition for space. A difference, however, in the thermal qualities of the upper and the lower basking areas, caused by their different microhabitat characteristics, also cannot be ruled out as a reason for the observed segregation.

After emergence, most individuals usually basked for two to three days, before leaving the basking area of the hibernaculum. Specimens of different species could often be observed together without any inter- or intraspecific aggressive behavior as found by Capula et al. (1997). It seems that the male *V. ammodytes* showed a tendency to remain longer in this area from its emergence, to the first shedding period, which takes place a few weeks after the

emergence (see Saint Girons 1979), a behavior common in European vipers (see Viitanen 1967, Prestt 1971; Phelps 2004). No post hibernation reproductive behavior by any of the species was ever observed at the basking area.

It seems that the timing of emergence for all species coincide, with the exception of *M. insignitus*. It could be expected that this thermophilic species (Blázquez 1995), which in southern Bulgaria is at the northern range of its distribution (Speybroeck et al. 2016), should emerge last, when the temperatures are higher. However, a tendency towards early emergence, at slightly lower temperatures, than the other species, followed by early dispersal from the hibernaculum, was present in this species. The period of emergence is reported to be correlated with the depth at which the snakes hibernate, either due to the structure of the den, making the emergence of the snakes from lower levels impossible, until all of those above them are out (Carpenter 1953), or due to the differences in the thermal gradient, with upper parts warming up earlier, thus snakes in those parts emerging first (Macartney et al. 1989). As stated above, the species in our studied hibernaculum probably hibernate separately, so blocked passages seem unlikely to cause difference in emerging periods. Differences in mating systems and mating periods are also considered to cause differences in emergence periods in snakes (Gregory 1984; Graves and Duvall 1990). Male snakes with vernal (spring) spermatogenetic cycle emerge earlier than male snakes with aestival (autumn) one, due to the prolonged basking period needed for the completion of spermatogenesis (Prestt 1971; Nilson 1980). Snakes with summer or autumn mating emerge later than snakes with spring mating, in order to wait for optimal temperatures (Graves and Duvall 1990). The mating periods of all five species in our hibernaculum are in spring (see Stojanov et al. 2011). Both *V. ammodytes* and *M. insignitus* have vernal spermatogenetic cycle (Saint Girons 1979; Feriche et al. 2008), but data for the other species are lacking. However, differences in mating systems should affect only the adult male specimens, but in *M. insignitus*, the subadults also emerged and left the hibernaculum before the other species. The spatial competition with the ecologically similar *D. caspius* may be considered as an alternate hypothesis for the early emergence of this species. Graves and Duvall (1990) stated that differences in emergence period may be related to different requirements and opportunities, encountered during the active season. After dispersing from the hibernaculum, the two species, which were equally abundant in the study area, used the same microhabitats. Between April and June the two could even be found on the exact same spots, although never together. These spots were a stone wall and an abandoned transformer substation, located 130 and 50 m away from the hibernaculum, respectively, and were used as temporary shelters during spring dispersal, as well as shedding sites in the active season. Some competition for those spots may exist between the two species. When one of the species was present at one of those places, the other was absent. A few days later a shift in occurrence was observed, with the latter species being present, and the first absent. Microhabitats, especially stony ones, may play an important role in the hierarchical selection of habitats by some snake species (see Reinert 1993; Kurek et al. 2018). Therefore, earlier emergence may give advantage to *M. insignitus* in this competition for space utilization. Furthermore, the earlier departure of this species from the hibernaculum probably reduces the competition in the basking area, since both *M. insignitus* and *D. caspius* mainly used the lower part of the hibernaculum in the initial days of basking, and both were presented with most individuals on it.

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